(54) Title: METHOD AND APPARATUS FOR THE MANIPULATION OF PARTICLES BY MEANS OF DIELECTROPHORESIS

(57) Abstract

The invention relates to an apparatus and a method for establishing closed dielectrophoretic potential cages and precise displacement thereof, suitable for the manipulation of particles and detection of same. The apparatus comprises a first array of selectively addressable electrodes, lying on a substantially planar substrate and facing toward a second array comprising one electrode. The arrays define the upper and lower bounds of a micro-chamber where particles are placed in liquid suspension. By applying in-phase and counter-phase periodic signals to electrodes, one or more independent potential cages are established which cause particles to be attracted to or repelled from cages according to signal frequency and the dielectric characteristics of the particles and suspending medium. By properly applying voltage signal patterns into arrays, cages may trap one or more particles, thus permitting them to levitate steadily and/or move. In the preferred embodiment, where one array is integrated on a semiconductor substrate, displacement of particles can be monitored by embedded sensors to achieve complex operations upon the sample to be analyzed, such as isolation, selection and precise counting of particles.

METHOD AND APPARATUS FOR THE MANIPULATION OF PARTICLES BY MEANS OF DIELECTROPHORESIS

FIELD OF THE INVENTION

An apparatus and method are disclosed for the manipulation and detection of particles such as cells, polystyrene beads, bubbles, and organelles by means of dielectrophoretic forces.

BACKGROUND OF THE INVENTION

Dielectrophoresis (DEP) relates to the physical phenomenon whereby neutral particles, when subject to nonuniform, time stationary (DC) or time varying (AC) electric fields, experience a net force directed towards locations with increasing (pDEP) or decreasing (nDEP) field intensity. If the intensity of the said dielectrophoretic force is comparable to the gravitational one, an equilibrium may be established in order to levitate small particles. The intensity of the dielectrophoretic force, as well as its direction, strongly depend on the dielectric and conductive properties of particles and on the medium in which the body is immersed. In turn, these properties may vary as a function of frequency for AC fields.

A description of the theory of dielectrophoresis has been published by H. A. Pohl in "Dielectrophoresis" Cambridge University Press (Cambridge 1978). A theoretical formulation of a case of particular interest is reported in Biochimica et Biophysica Acta 1243 (1995) p. 185-194, and Journal of Physics, D: Applied Physics, 27 (1994) pp. 1571-1574.

Studies on the action of dielectrophoresis on both biological matter (cells, bacteria, viruses DNA, etc.) and inorganic matter particles have lately proposed using DEP forces for the isolation of elements from a mixture of microorganisms, their

characterization by differences in physical properties and their general manipulation. For such purposes, the suggestion has been to utilize systems of the same scale of particle size, in order to reduce the potentials required by electrical field distributions.

U.S. Pat. 5,888,370, U.S. Pat. 4,305,797, U.S. Pat. 5,454,472, U.S. Pat. 4,326,934, U.S. Pat. 5,489,506, U.S. Pat. 5,589,047, U.S. Pat. 5,814,200, teach different methods of separating particles in a sample, based on differences in dielectric and conductive properties characterizing the species they belong to. The main drawback, common to all devices proposed resides in the requirement of mechanical and fluid dynamic microsystems for moving fluids within the system. Moreover, each apparatus of the above listed patents involves contact and friction of particles with the surfaces of the system, compromising their mobility and integrity.

U.S. Pat. 5,344,535 teaches a system for the characterization of microorganism properties. The disclosed apparatus and the proposed method have the shortcoming of providing data on a large number of bodies, lacking the advantages of analysis on a single particle. In addition, the disclosed system is unable to prevent contact of particles with device surfaces.

U.S. Pat. 4,956,065 teaches an apparatus to levitate single particles and analyze their physical properties. However, this device requires a feedback control system since it employs pDEP. Moreover, the system is unsuitable for miniaturization, having a three-dimensional topology which is not compatible with mainstream microelectronic fabrication technologies.

The paper by T. Schnelle, R. Hagedorn, G. Fuhr, S. Fiedler, T. Muller in "Biochimica et Biophysica Acta", 1157(1993) pp. 127-140, describes research and experiments on the creation of three-dimensional potential cages for the manipulation of particles. However, the proposed structures are very difficult to fabricate in scale with the size of cells (required for trapping a single cell in the cage). In fact,

the major problem of these systems is the vertical alignment of two structures on a micro-metric scale.

SUMMARY OF THE INVENTION

The present invention relates to a method for the stable levitation and independent motion of neutral particles in a liquid suspending medium and their precise displacement by means of an electronically programmable device adapted to receive such a solution.

As used above, the term "particle" is intended to include biological matter such as cells, cell aggregates, cell organelles, bacteria, viruses and nucleic acids as well as inorganic matter such as minerals, crystals, synthetic particles and gas bubbles. By "dielectrophoretic potential" what is meant is a three-dimensional (3D) scalar function whose gradient is equal to the dielectrophoretic force. By "equipotential surface" what is meant is a surface defined in the 3D space whose points have the same dielectrophoretic potential; the dielectrophoretic force is always perpendicular to said surface. By "potential cage" what is meant is a portion of space enclosed by an equipotential surface and containing a local minimum of the dielectrophoretic potential. By "particle trapped inside a potential cage" what is meant is a particle subject to dielectrophoretic force and located inside the said cage. At equilibrium, if the particle is subject to dielectrophoretic force only, then it will be located at a position corresponding to the said dielectrophoretic potential minimum, otherwise it will be positioned at a displacement from that minimum given by the balance of forces.

The preferred, but not exclusive, embodiment of the present invention, comprises two main opposed modules; the first one comprises a plurality of electrically conductive electrodes, whose shape may be of various types, regularly arranged on a insulating substrate; the electrodes may be optionally coated with an insulating layer

protecting them from charge carriers present in the liquid suspension. If this module is realized with integrated circuit fabrication technology, it may include memory elements for electrode programming, configurable signal generators such as sine or square wave, impulse etc., with variable frequency and phase, any integrable sensor device for detecting the presence of the particle, input/output circuits etc.. The second module comprises a single large electrode fabricated in a conductive, optionally transparent matter, which in turn may be coated with an insulating layer. It is to be understood that this large electrode may also be split into several electrodes, if desired. A spacer can be inserted between the first (lower) module and the second (upper) one in order to implement a chamber for the containment of the sample to be analyzed or manipulated. The same spacer may also serve to establish separation walls inside the device so as to realize multiple chambers. Of course, the spacer may also be integrated in either the first or second module, or both. Finally, a visual inspection system such as a microscope and camera may be added to the device, as well as fluidics systems for moving liquid or semi-liquid matter in and out of the device.

The architecture of the apparatus described allows one, by simply applying in-phase and counter-phase periodic signals to the electrodes, to establish in the micro-chamber one or more independent potential cages, the strength of which may be varied by acting on the frequency as well as on the amplitude of the signals applied. The cages may trap one or more particles, thus permitting them either to levitate steadily or to move within the micro-chamber, or both. Due to this feature, any contact or friction of the particles with the chamber borders and the electrodes can be avoided. The height and relative displacement of cages can be independently set by an appropriate choice of signals and does not require any mechanical adjustment. Thus, the device can be configured as a fully programmable electronic apparatus.

The methodology for the displacement of the potential cage along the micro-

chamber is much like the principle used in charge coupled devices (CCDs). For example, if a first electrode is in-phase with the upper module and is surrounded by electrodes connected to counter-phase signals, a potential cage is established on top of it. Then, by simply applying in-phase signals to one of the adjacent electrodes (in the same direction as the programmed motion) the potential cage spreads over the two electrodes thus aligning its center in between them: the particle has thus moved half of the cell-pitch. Once the transient has expired the phase is reversed for the first electrode (where the particle was located at the beginning of the phase): this causes the potential cage to shrink and to move on top of the in-phase electrode which is displaced one cell-pitch away from the previous electrode. By repeating the latter operation along other axis any potential cage may be moved around the array plane.

The shortcomings of devices known from the prior art can be overcome thanks to the apparatus according to the present invention, which allows one to establish a spatial distribution of electric fields that induce closed dielectrophoretic potential cages. The proposed device does not require precise alignment of the two main modules, thus optimizing both simplicity and production cost: it overcomes most of the restrictions related to the implementation cost and to the minimum allowable cage potential size inherent in the prior art (alignment gets more and more critical as the electrode size shrinks). Hence misalignment of the two main modules does not compromise the system functionality. The importance of this feature may be better appreciated if one thinks of all the applications in which the device is manually opened and/or closed, requiring repeated and flexible use; it may thus be implemented in low-cost, standard manufacturing microelectronic technology. Moreover, the proposed device easily allows trapped particles to be displaced along a wide range compared to the particle size.

In addition, no prior art system that employs fluidics or "traveling fields" for the

displacement of particles achieves precise particle positioning while keeping particles away from device surfaces; yet, it is apparent that such a result can be achieved if three-dimensional potential-cages positioned at a fixed height and movable along other directions of the apparatus are available. Further advantages of the invention stem from the possibility to control the height of the cage potentials by adjusting the voltage values applied.

Thanks to the flexible programming of the disclosed invention, virtual paths can be established, thus avoiding the need for application-specific devices and widening the range of potential applications and users. Furthermore, the ability to integrate optical and/or capacitive sensing allows one to overcome the need for bulky detection instrumentation normally used in this field, such as microscopes and cameras, although it does not prevent it form being used for visual inspection of the internal micro-chamber. Processing the integrated sensors information with feedback control techniques, enables complex operations to be carried out in a fully automated way: for example, characterization of the physical properties of particles under test.

Finally, the closed potential cage approach prevents particles from getting out of control in the presence of: hydrodynamic flows due to thermal gradients, significant Brownian motions (equally likely from any direction), or forces due to Archimedes' balance. In fact, in all the above cases, any apparatus providing non-closed potential surfaces proves ineffective, since it cannot counterbalance upward forces.

Some unique features of the apparatus according to the present invention, as compared to those present in the prior art, may be summarized as:

1. the capability of establishing closed dielectrophoretic potential cages without requirements of alignment between modules, whereby single or groups of particles are independently trapped in the cages and placed in stable suspension by means of dielectrophoretic forces without any friction with electrodes or boundaries.

2. The ability to move any potential cage independently around the micro-chamber by virtue of electronically programmed electric signals.

3. The possibility of shrinking the cage size according to application requirements and implementation, thus permitting fabrication of the device in microelectronic technology with implementation of embedded sensors, actuators and signal generation.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic three-dimensional view of a part of the device devoted to sample manipulation, with the modular structure formed by the substrate, including the electrodes, and the lid;

FIG. 2 shows a detailed cross-sectional view of the same structure as in FIG. 1;

FIG. 3 shows an embodiment of the electrode arrangement ;

FIG. 4 shows an alternative embodiment of the electrode arrangement;

FIG. 5 shows a blow-up schematic diagram of the device emphasizing the presence of a third module;

FIG. 6 shows a three-dimensional surface in which each point has the same root mean square (RMS) electric-field magnitude;

FIG. 7 shows the same plot as in FIG. 6 for a different set of signals applied;

FIG. 8 sketches the cage motion principle highlighting the fundamental steps and their timing;

FIG. 9 shows a 2-D plot of the RMS magnitude of the electric field on a vertical section orthogonal to the electrodes, assuming that electrodes extend for the whole device length;

FIG. 10 shows the same plot as in FIG. 9 for a different set of voltages applied;

- 8 -

FIG. **11** shows a plot of the absolute value of the gradient of the square RMS magnitude of the electric field along a horizontal cross section of the plot in FIG. **9** passing through the dielectrophoretic potential minimum ($4.3\mu m$ above the electrode surface);

FIG. **12** shows a plot of the absolute value of the gradient of the square RMS magnitude of the electric field, along a vertical section of the plot in FIG. **9** passing through the dielectrophoretic potential minimum for different values of the voltage applied to the upper electrode;

FIG. **13** shows a plot of the absolute value of the gradient of the square RMS magnitude of the electric field, along an horizontal cross section of the plot in FIG. **10** passing through the dielectrophoretic potential minimum;

FIG. **14** shows a plot of the absolute value of the gradient of the square RMS magnitude of the electric field, along a vertical section of the plot in FIG. **10** passing through the dielectrophoretic potential minimum;

FIG. **15** shows a simplified block diagram of the first substrate;

FIG. **16** sketches the block diagram of a cell in the array;

FIG. **17** sketches the measurement instruments which may be interfaced with the apparatus;

FIG. **18** shows a schematic plot of the nDEP potential along a generic section, comparing cage size with particle one;

FIG. **19** sketches a special electrode layout which enables one to optimize the area available for the electrode programming circuit;

FIG. **20** sketches a special electrode layout which allows for optimization of the area available for the electrode circuitry relating to a specific embodiment targeted to particle counting;

FIG. **21** shows an embodiment of an integrated optical sensor;

FIG. **22** shows an embodiment of an integrated capacitive sensor;

FIG. 23 shows an embodiment of an integrated capacitive sensor;

DETAILED DESCRIPTION

The features and advantages of the invention will be clearer from the description of embodiments illustrated by examples in what follows. It is to be understood that examples used herein are for purpose of describing a particular embodiment and are not intended to be limiting of the spirit of the invention.

Dielectrophoretic potential energy

A dielectric sphere immersed in a liquid at coordinates (x, y, z), and subject to the effect of spatially non-uniform AC or DC electric fields, is subject to a dielectrophoretic force $F(t)$ whose time-averaged value is described by the following:

$$\langle F(t) \rangle = 2\pi \epsilon_0 \epsilon_m r^3 \left\{ Re\left[f_{CM}\right] \nabla \left(E_{RMS}\right)^2 + \right.$$
$$\left. + Im\left[f_{CM}\right] \left(E_{x0}^2 \nabla \varphi_x + E_{y0}^2 \nabla \varphi_y + E_{z0}^2 \nabla \varphi_z \right) \right\} \tag{1}$$

where ϵ_0 is the vacuum dielectric constant, r is the particle radius, E_{RMS} is the root mean square value of the electric field, E_{x0}, E_{y0}, E_{z0} are the electric field component along axes x, y, z, while $\varphi_{x,y,x}$ are the phases of the electric field component and f_{CM} is the well known Clausius-Mossotti factor defined as:

$$f_{CM} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}$$

where ϵ_p^* and ϵ_m^* represent the relative complex permittivity of the particle and of the suspending medium respectively, defined as: $\epsilon_{m,p}^* = \epsilon_{m,p} - i\sigma/(\epsilon_0\omega)$, where ϵ is the relative dielectric constant, σ is the conductivity, ω is the angular frequency and i is the square root of minus one.

If electric field phases are constant, equation (1) may be simplified to:

$$\langle F(t) \rangle = 2\pi \epsilon_0 \epsilon_m r^3 Re\left[f_{CM}\right] \nabla (E_{RMS})^2 \qquad (2)$$

where nDEP is defined by $Re[f_{CM}] < 0$ while pDEP is defined by $Re[f_{CM}] > 0$. For high values of ω, where $\epsilon_m^*, \epsilon_p^* \leftarrow \epsilon_m, \epsilon_p$ pDEP is established on a particle whenever $\epsilon_m < \epsilon_p$ whilst nDEP is established whenever $\epsilon_m > \epsilon_p$. Since $\epsilon_{m,p}^* = \epsilon_{m,p}^*(\omega)$, thus $f_{CM} = f_{CM}(\omega)$ so that $Re[f_{CM}]$ may have different signs for different species of particle at a given frequency. The method of choosing an angular frequency ω so that two different species of particles experience nDEP and pDEP respectively, is commonly used as known art for selection purposes.

Since the force described in equation (2) is conservative, it is possible to define the dielectrophoretic potential energy:

$$\langle W \rangle = -2\pi \epsilon_0 \epsilon_m r^3 Re\left[f_{CM}\right](E_{RMS})^2,$$

where,

$$\langle F(t) \rangle = -\nabla \langle W \rangle,$$

If the voltage signals applied to electrodes and establishing the electric field are periodic, it can easily be shown that

$$\langle W \rangle = -\alpha 2\pi \epsilon_0 \epsilon_m r^3 Re\left[f_{CM}\right] E^2 \qquad (3)$$

where α is a constant that depends on the shape of the voltage signals applied to electrodes and E is the magnitude of the electric field, (e.g. $\alpha = 1$ for square-wave signals and $\alpha = 1/\sqrt{2}$ for sinusoidal signals). Thus, minima of E^2 are also minima of the negative dielectrophoretic potential (since for nDEP, $Re[f_{CM}] < 0$) as well as

maxima of the positive dielectrophoretic potential (since for pDEP, $Re[f_{CM}] > 0$).

In what follows, "dielectrophoretic potential" will be used as a synonym of "negative dielectrophoretic potential". Furthermore, since E^2 is a monotonic function of E, the minima or maxima of E correspond to the minima or maxima of the dielectrophoretic potential function $\langle W \rangle$. This is very useful since the location of the dielectrophoretic potential minima or maxima can be found by time-stationary simulations of the electric field as illustrated by the figures enclosed. To summarize the above concept, it can be easily demonstrated that:

> *any dielectrophoretic potential cage (containing nDEP potential energy*
> *local minima) is enclosed by at least one imaginary closed surface com-*
> *posed of points of the space having constant electric field magnitude.*

If the spherical and homogeneous particle is subject to the gravitational force:

$$F_g = \frac{4}{3}\pi R^3 \Delta \rho \, g$$

where $\Delta \rho$ is the mass density difference between the particle and the medium and g is the acceleration of gravity ($9.807\, m/s^2$), as well as to nDEP, then stable suspension is achieved according to:

$$\langle F(t) \rangle > F_g. \tag{4}$$

Since the relative dielectric constant cannot be greater than unity (e.g. if the particle is a bubble of air immersed in water, where $\epsilon_p = 1$ and $\epsilon_m \simeq 81$), then the minimum value of ∇E_{rms}^2 required for balancing the gravitational force acting on the particle can be estimated, by using equation (4), as $1.835 \cdot 10^3 (V/cm)^2/\mu m$ which is achievable by using standard microelectronic technology and/or micro-machining techniques. Again, particles that are twice as heavy than water ($\Delta \rho \simeq 1000\, Kg/m^3$)

can be suspended in water, if the relative dielectric constant of the medium is at least $2.2 \div 20.3$ times greater than that of the particle for typical values of ∇E^2_{rms}.

General structure of the device

The apparatus according to the preferred embodiment comprises two main modules. The first module **A1** (FIG. 1) comprises an array **M1** of selectively addressable electrodes **LIJ** (FIG. 1 and 2) being disposed upon an insulating substrate **O1**, grown on a semiconductor substrate **C** (FIG. 1 and 2). The second module **A2** is made up of a single large electrode **M2** which is fabricated on a substrate **O2** (FIG. 1 and 2) and is opposed to the said array **M1**. In between the two modules a micro-chamber (**L** in FIG. 1 and 2) is formed, containing the particles (**BIO** in FIG. 1) in liquid suspension. Methods for containing the liquid suspension in the micro-chamber will be described later on. The first module **A1** is made in silicon, according to known microelectronic technology, or any other suitable substrate materials, such as glass, silicon dioxide, plastic, or ceramic materials. An electrode may be of any size, preferably ranging from sub-micron ($\sim 0.1\mu m$) to several millimeters (mm) with $5\mu m$ to $100\mu m$ being the preferred size range for devices fabricated using micro-lithographic techniques, and $100\mu m$ to $5mm$ for devices fabricated using micro-machining and/or printed circuit board (PCB) techniques. The device can be designed to have as few as under ten electrodes or as many as thousands or millions of electrodes. The distance **DL** between the two modules may vary according to the embodiments but is preferably in the order of magnitude of the electrode size **DE** (FIG. 2).

Electrodes can be coated by an insulating layer (**R1** in FIG. 2) to prevent electrolysis due to the interaction of electrodes with the liquid medium,which may contain a high concentration of positive and negative ions. Such a layer may be avoided if either the electrodes are composed of material that does not chemically react with the liquid medium or the frequency of signals energizing electrodes is high enough

to make electrolysis negligible. Finally, some circuitry, the purpose of which will be explained later in greater detail, may be placed underneath each electrode.

Array electrodes may be of any shape, depending on the effect to be achieved; for example's sake, an array **M1** of square electrodes are shown in the preferred embodiment of FIG. 1, while FIG. 2 shows a cross-section of electrodes emphasizing their width and relative displacements (**DE** and **DO**).

In an alternative embodiment, electrodes may be of hexagonal shape (as illustrated in FIG. 3), which allows the number of electrodes to establish a single potential cage to be reduced from 9 to 7 (as will be shown later) and offers a larger number of possible cage motion directions **DIR** (from 4 to 6).

The second main module **A2** comprises a single large electrically conductive electrode (**M2** in FIG. 1 and 2) which is opposed to the first module **A1**. It also serves as the upper bound of chamber **L** containing the liquid suspension of particles. This electrode may be coated with an insulating layer (**R2** in FIG. 2) to protect it against electrolysis and may have a mechanical support (**O2** in FIG. 1 and 2). In the preferred embodiment, this electrode is a single, planar surface of conductive glass, thus permitting visual inspection of the micro-chamber.

A spacer **A3** (FIG. 5) is used to separate the two modules (**A1** and **A2** in FIG. 5, in which **A1** comprises **R1**, **O1**, **M1** and **C**, while **A2** comprises **R2**, **O2**, **M2**) by a given distance (**DL** in FIG. 2). The spacer may also be used to contain the sample for manipulation or analysis.

By applying appropriate time-varying signals to different subsets of electrodes, a potential cage **S1** (FIG. 1 and FIG. 6) that may contain one or more particle **BIO** is established upon one or more electrode. The potential cage is located at some height above the array plane, the value of which depends on the signals applied, on the ratio of electrode size **DE** and pitch **DO** and on the distance between the two modules **DL**. By changing the subset of electrodes to which signals are applied,

one or more potential cages may be moved around micro-chamber **L** in a direction parallel to the electrode array.

From simulation results, emerges that, for constant values of size **DL**, the greater the ratio between size **DE** and **DO**, the better the properties of the cage in terms of DEP force strength.

Method for establishing potential cages

In order to establish potential cages on top of a single electrode, a pattern of voltage signals is applied to corresponding subsets of electrodes. FIG. 4 illustrates a set of electrodes **L1-L12** in array **M1**, used as a reference for numerical simulations.

Defining:

$$V_{sq}\left(\omega t,\ \varphi\right) = \begin{cases} 1 & \text{if } \cos\left(\omega t + \varphi\right) > 0 \\ -1 & \text{if } \cos\left(\omega t + \varphi\right) \leq 0 \end{cases}$$

as a square wave signal having period T, where $\omega = 2\pi/T$, the following voltage signals are applied to electrodes:

$$V_{La} = V_e \cdot V_{sq}\left(\omega t,\ \varphi\right) \qquad \forall\, a \in \{1-6, 8-12\}$$

$$V_{L7} = V_e \cdot V_{sq}\left(\omega t,\ \varphi + \pi\right)$$

$$V_{M2} = V_c \cdot V_{sq}\left(\omega t,\ \varphi + \pi\right)$$

where V_{La}, $a \in \{1-12\}$ are signals applied to electrodes **L1-L12**, V_{M2} is the voltage signal applied to **M2**, and V_e and V_c are constant values. Using voltage

patterns as indicated above, the electric field phases are constant, so that equation (2) applies. Hence, the numerical simulations of the electric field magnitude will be used to verify the establishing of dielectrophoretic potential cages.

FIG. 6 shows the result of a numerical simulation regarding the same set of electrodes as illustrated in FIG. 4 energized by the above mentioned voltage signal patterns where: $\mathbf{DE} = 5\mu m$, $\mathbf{DO} = 1\mu m$, $\mathbf{DL} = 10\mu m$, $V_e = 2.5V$, $V_c = 0.V$. Water is chosen as the liquid medium between the modules $\mathbf{A1}$ and $\mathbf{A2}$, with $\epsilon_m \simeq 81$. $\mathbf{R2}$ is negligible and $\mathbf{R1} = 1\mu m$. The plot in FIG. 6 shows a 3D environment containing a closed surface whose points are characterized by having a constant electric field magnitude ($\mathbf{S1}$ in FIG. 6) at 400V/cm. This proves, by virtue of equation (3), that the dielectrophoretic equipotential surface is likewise closed, hence a potential cage is established on top of $\mathbf{L7}$. Thus, a pattern of only two signals, having the same frequency and counter-phase relationship, is needed to establish a minimum of the dielectrophoretic potential function on top of $\mathbf{L7}$. From simulation it also emerges that by increasing $V_c \in [-2.5, \ 2.5]\ V$ the dielectrophoretic forces of the cage increase, while the cage height decreases with respect to the array plane. In the preferred embodiment, in which square electrodes are employed, the minimum number of array electrodes for establishing a single dielectrophoretic potential cage is 9 ($\mathbf{L2}$-$\mathbf{L4}$, $\mathbf{L6}$-$\mathbf{L8}$, $\mathbf{L10}$-$\mathbf{L12}$ in FIG. 4). On the other hand, if a hexagonal array of electrodes is employed, as illustrated in FIG. 3, the minimum number of array electrodes for establishing a single dielectrophoretic potential cage is 7, such as electrodes $\mathbf{E1}$-$\mathbf{E7}$.

In order to establish potential cages at a mid point on top of two electrodes, a different pattern of voltage signals is applied to corresponding subsets of electrodes. FIG. 7 shows the result obtained when the stimuli applied to the electrodes are as follows:

$$V_{La} = V_e \cdot V_{sq}(\omega t, \varphi) \qquad \forall\, a \in \{1-5, 8-12\}$$

$$V_{L6} = V_{L7} = V_e \cdot V_{sq} \left(\omega t, \; \varphi + \pi \right)$$

$$V_{M2} = V_c \cdot V_{sq} \left(\omega t, \; \varphi + \pi \right),$$

where all the other parameters are the same as before. S2 in FIG. 7 again shows a closed surface whose points have a constant electric field strength at 400V/cm, where the center is, however, located on top of the mid point between electrodes L6 and L7.

This last pattern of voltage signals, in combination with the previous one, can be used for moving potential cages in a programmed direction. More specifically, by repeatedly changing the subsets of electrodes to which in-phase and counter-phase signals are respectively applied, in particular by alternating and shifting the two patterns described in a given direction, it is possible to move the potential cage in that direction. As an example, FIG. 8 sketches three plots where the potential cage is moved from a position on top of L7 to another position on top of L6: the first at time T1, the second at T2 and the third at T3. In each plot the phase of electrodes L5, L6, L7, L8 is reported, showing the moving-cage principle. With increasing time, the electrode with phase $\varphi + \pi$ shifts along a decreasing X direction in two steps: at T2 electrode L6 is connected to a signal having phase $\varphi + \pi$ which is the same as L7 and then, at time step T3, the phase of L7 is reversed.

Obviously, the time interval between switching phases should be carefully chosen according to system characteristics: force intensity, fluid medium viscosity, particle size, etc.. For this purpose it may be useful to employ embedded sensors to detect the presence/absence of one or more particles in each position so that the time distance

can be adjusted according to sensor data.

To illustrate the capability of the invention to move closed dielectrophoretic cages, FIG. **9** and **10** show 2-D simulations of the electric field distribution along a cross section of the device. When the voltages applied to electrodes **P1, P2** and **P3,** and the lid electrode **M2** are:

$$V_{Pa} = V_e \cdot V_{sq}\left(\omega t,\ \varphi\right) \qquad \forall\, a \in \{1, 3\}$$

$$V_{P2} = V_e \cdot V_{sq}\left(\omega t,\ \varphi + \pi\right)$$

$$V_{M2} = V_c \cdot V_{sq}\left(\omega t,\ \varphi + \pi\right)$$

where, $V_e = 2.5V$ and $V_c = 0$, the resulting electric-field distribution is as shown in FIG. **9,** in which the darker regions **S3** mean a lower electric-field magnitude, while the brighter regions mean a higher electric-field magnitude.

FIG. **11** shows a plot (in log scale) of the absolute value of the gradient of the square electric field magnitude, taken along a horizontal cross section of the plot of FIG. **9** passing through the center of the cage ($4.3\mu m$ above the array surface). This kind of plot is very useful since the values of the plots are directly proportional to the dielectrophoretic force, from which one can pinpoint the location of the minimum dielectrophoretic potential (where dielectrophoretic forces are equal to zero). FIG. **12** shows a similar plot taken along a vertical cross section of the plot of FIG. **9** including the center of the potential cage for different values of V_c, ranging from +2.5V to -0.5V.

In order to establish a dielectrophoretic potential cage in the region above the mid point between **P2** and **P3,** the following voltages can be applied:

$$V_{P1} = V_e \cdot V_{sq}(\omega t, \varphi)$$

$$V_{P2} = V_{P3} = V_e \cdot V_{sq}(\omega t, \varphi + \pi)$$

$$V_{M2} = V_c \cdot V_{sq}(\omega t, \varphi + \pi)$$

where $V_e = 2.5V$ and $V_c = 1.5V$. The result is shown in FIG. 10 where S4 is the region in which the potential cage is located.

FIG. 13 shows a plot of the absolute value of the gradient of the square electric field magnitude, along a horizontal cross section of the plot in FIG. 10 including the cage center, in the case of $V_c = 1.5V$; the height of the cage center from the array surface is $4.3\mu m$. The presence of two values with gradient equal to zero in FIG. 13 is due to a maximum on top of electrode P1 and to a minimum located in the region above the mid point between P2 and P3. A given particle subject to such a dielectrophoretic force field would find a stable equilibrium point at the aforesaid minimum and an unstable equilibrium point at the aforesaid maximum. FIG. 14 shows a similar plot taken along a vertical cross section of the plot of FIG. 10 passing through the cage center, in the case of $V_c = 1.5V$.

To summarize, the establishing of dielectrophoretic potential cages, as disclosed by the present invention, can be achieved by using a pattern of as few as two voltage signal having the same frequency and counter-phase relationship. Furthermore, movement of such cages along a guide path parallel to the array surface can be achieved by simply selecting convenient patterns of subsets of electrodes to which

apply the two above mentioned signals at different time steps. The electrode voltage waveforms may either come from on-chip oscillators or from external generators.

Preferred embodiment: integration on semiconductor substrate

A schematic diagram of the first module **A1** in the preferred embodiment is illustrated in FIG. **15**. A silicon substrate embeds an array **M3** of micro-locations **EIJ** that are independently addressed by proper addressing circuits, **DX e DY**, by means of a number of electrical communication channels running along vertical lines **YJ** and horizontal lines **XI**. The module communicates with external signals **XYN** by means of an interface circuit **IO**, which in turn communicates by means of connection **CX** and **CY** with addressing circuits **DX e DY**, and by means of a set of connections **CS** controls the waveform generation and sensor readout circuit **DS** for delivering the signal to be applied to the micro-locations **EIJ** and for collecting signals from the sensors in the micro-locations by means of connections **FS**. The apparatus is connected with a number of fluidic communication channels **FM** with the external means **IS** for the management of liquid suspension medium containing the particles. Various instruments can be used for interfacing to the device **SS** by means of electrical communication channels **XYN** such as: computer, external waveform generators, analyzers etc. (**WS** in FIG. **17**), and by means of fluidic dynamic channels, such as micro-pumps **IS** and by means of optical channels **OC** such as microscope, camera, etc. **MS**.

In the preferred embodiment each micro-location **EIJ** (FIG. **16**) comprises at least one electrode **LIJ** to be energized by the electrical signals, a circuit for the electrode signal management **MIJ** (FIG. **16**) and a sensor **SIJ** to detect the presence/absence of particles on top of each cell. Each of these blocks may communicate with others inside the same element by means of local connections **C1**, **C2**, **C3**. Moreover the circuit for electrode signal management (**MIJ** FIG. **16**) can communi-

cate with external circuits by means of global connections **XI** and **YJ**. The circuit **MIJ** may contain switches and memory elements suitable for selecting and storing the routing of pattern signals to electrode **LIJ**. Since two voltage signal patterns are sufficient for establishing and moving dielectrophoretic potential cages, as explained in the previous section, one electronic memory means is sufficient to determine whether the electrode will be connected to the in-phase or to the counter-phase signal. To optimize the space available, various different arrangements of **LIJ**, **SIJ** and **MIJ** are possible: for example **LIJ** may entirely overlap **MIJ** and partially cover **SIJ** or simply be placed beside **SIJ** according to the microelectronic technology rules.

A peculiar characteristic of the present invention considered to be unique from prior art dielectrophoretic devices, consists in its ability to integrate on the same substrate both actuators, for biological particle manipulation, and sensors for detection of particles. Some indicative but not exclusive examples of integrated sensors are shown in FIG. **21, 22** and **23**.

FIG. **21** sketches an implementation of a sensing scheme using an optical sensor to detect the presence/absence of a biological particle **BIO**. If the lid **A1** is made of transparent and conductive material, a window **WI** can be opened on the electrode **LIJ**. The size of **WI** is negligible for modifying the dielectrophoretic potential but large enough to permit a sufficient amount of radiation to impinge onto the substrate. Underneath **LIJ** a photo-junction **CPH** working in continuous or storage mode is realized into substrate **C** according to known art. The presence/absence of the biological element **BIO** determines the amount of optical energy reaching the photodiode, causing a change of charge accumulated across **CPH** during the integration time. This variation is detected by a conventional charge amplifier **CHA** composed of an amplifier **OPA**, a feedback capacitor **CR** and a reference voltage source **VRE**. The connection to this charge amplifier is established by enabling a switch **SW1** after switch **SW2** has been opened, thus permitting the accumulated

charge to be integrated onto **CR**. The photodiode and charge amplifier are designed, according to known art, to obtain a signal to noise ratio sufficient to detect the presence/absence of the biological particle. As an example, with reference to a structure with the dimensions previously described for simulations, and assuming a $0.7\mu m$ CMOS technology, we may consider a photodiode of $1 \times 2\mu m$ in the substrate under the electrode. Analyzing the signal to noise ratio according to known art, a variation of 10% of the particle transparency with respect to the liquid medium can be revealed using integration times larger than $3\mu s$.

In another embodiment, capacitive sensing is used as sketched in FIG. **22**. A voltage signal **SIG** applied to the lid **A1** induces a variation in the electric field **ELE** between **A1** and **LIJ**. The corresponding capacitance variation can be detected by a charge amplifier **CHA** similar to the case of optical sensing.

In FIG. **23** another implementation of capacitive sensing is sketched, using two electrodes **FR1** and **FR2** coplanar to element **LIJ**. A voltage signal **SIG** applied to the element **FR1** determines a variation in the fringing electric field **ELE** towards **FR2**. The interposition of biological element **BIO** in the region affected by this electric field causes a variation in the capacitance value between **FR1** and **FR2**. This variation is detected by a charge amplifier **CHA** similar to the previous sensing schemes. The electrodes **FR1** and **FR2** may be omitted if the elements **LIJ** of the adjacent locations are used in their place. It is to be understood that more than one of the above described sensing principles may be used in the same device to enhance selectivity. As an example, different particles having the same transmissivity but a different dielectric constant, or having the same dielectric constant and different transmissivity may be discerned, by using a combination of capacitive and optical sensors.

An outstanding feature believed to be characteristic of the present invention is the

possibility to isolate single microorganisms of a size within the micron or sub-micron range, and to do so on a large number of them; indeed the size of microorganism which can be isolated will shrink following the advances in standard microelectronic fabrication technologies, in line with the shrinking in the minimum feature sizes that is characteristic of the technology. Indeed, if the size of the dielectrophoretic potential cage is small enough, no more than one particle of a given size may be trapped inside the cage. In order to better understand this feature of the device one can consider the distribution of the dielectrophoretic potential P (FIG. 18) along a horizontal cross section passing through the center of the cage, as established by the method disclosed, which has the typical behavior shown in FIG. 18 where two local maxima represent the borders of the cage potential along direction X. If the relative distance DP is twice the particle radius R to be isolated, then only one of the particles of the neighborhood will find room in the cage, so that if the cage is already occupied by a particle, an outward net force is exerted on other candidate particles, thus moving excess particles into either empty neighborhood cages or lateral reservoirs designed to contain the overspill particles. It is to be noted that if the above operation needs to be applied to all particles of the sample, the particle density should be smaller than the cage density.

The dielectrophoretic cage size is solely limited by the area dedicated to the circuitry of each electrode, which in turn depends on the technology adopted. To overcome this limit, a different electrode arrangement may be used, as disclosed in what follows, in which alternative electrode topologies are employed that are less flexible but more optimized with respect to potential cage size and targeted to applications requiring greater sensitivity such as sub-micron microorganism manipulation and counting. For applications requiring potential cages smaller than the area needed by electrode circuitry, alternative embodiments may be employed in order to achieve better area optimization.

As an example, in order to increase the area available for circuitry by 25%, it is feasible, using the same arrangement of electrodes, to connect an electrode LN (FIG. 19) out of a cluster of four LL to a fixed voltage signal pattern (for example to the in-phase one). From now on, we will refer to electrodes of type LN as "non-programmable electrodes" since they cannot be switched among the various voltage signal patterns but are tied to a fixed one. The above embodiment has the shortcoming of restricting the motion of potential cages solely along guide paths DR. On the other hand, the electrode arrangement shows the advantage of saving area for circuitry due to the fact that MIJ and SIJ blocks are not implemented in non-programmable electrodes LN.

Another alternative embodiment which further exploits the method for shrinking cage size at the expense of device flexibility is disclosed in FIG. 20. In this case the direction of motion is reduced to one dimension, along guide paths DR, and the cells SI (FIG. 20), designed for sensing the presence and possibly the type of particles, are arranged along one column SC, orthogonal to the allowed motion direction. Using proper signals, potential cages are regularly established along rows and moved along the guide paths DR throughout the column SC into a chamber CB designed to contain the particles whose number (and possibly type) has already been detected. Since motion directions along vertical guide paths are not used, non programmable electrodes LN are floor planned to save area available for cell circuitry. Hence, the area available for cell circuitry and for sensors is optimized since only one electrode in two needs to be programmed, and only cells SI need to integrate a sensor. The main shortcoming of this last alternative embodiment as compared to the preferred one resides in the longer time required for detecting the particles in the sample, since it depends on the number of row cells that particles must step through before reaching the sensors. On the other hand, the latter alternative embodiment can achieve smaller cage size, thus counting smaller particles.

Another approach according to the present invention is that of estimating the number of particles smaller than feasible cage size by taking advantage of sensors whose output is proportional to the number of particles contained into a cage. In using this method, cage size does not need to be set to minimum since the total number of particles can be estimated by summing the number of them in each cage, even if the the latter contain a plurality of particles. The main drawback of this approach is that the output of the sensors is designed to depend only on the number of particles, regardless of their type, so that their type cannot be detected.

Once the sample is inserted into the device -by means and instruments known to those with ordinary skill in the art such as micro-pump syringes etc., in fully automated or manual mode depending on user requirements -it is possible to work at the frequency with which one or more species of microorganisms are subject to negative dielectrophoresis; thus it is possible to trap the aforementioned biological objects into the dielectrophoretic potential cages and move them in longer or shorter paths around the device. The proposed device has the novel feature of moving the particles in suspension within the liquid instead of moving the liquid itself, thus reducing the need for complex and expensive fluidics procedures, enabling selected bodies to accumulate in proper sites or chambers and preventing the particles from being stressed by friction and collision. During the modes of operation described so far, the embedded sensors can monitor the presence of particles, thus providing for adaptive control of the device and its functionality in a feedback loop.

One important operation the device can perform is to characterize a sample of particulate and solubilized matter by differences in the physical properties of either the population or its components. This can be achieved by using the feature of guided cages, the mobility and strength of which depend on the physical properties and morphology of the biological matter being analyzed such as size, weight,

polarizability and conductivity, which will vary from species to species.

With its unique feature of inducing independent movement of one or more par-
ticles trapped in potential cages along guide paths, the device may easily be pro-
grammed to achieve several tasks: e.g. to separate one kind of microorganism from
a mixture of species by using their physical, dielectric and conductive properties.
Another possible application of the proposed device consists of making two or more
microorganisms collide by first trapping the objects in different cages and then mov-
ing them towards the same location of the device. As an example of the wide range of
application afforded by the device according to the present invention, various differ-
ent methods for manipulating particles are hereinafter disclosed, though again with
the proviso that examples used herein are not intended as limiting the spirit of the
invention.

It is envisioned that alternate or equivalent configurations of the present inven-
tion may be adopted without any restriction of the general invention as portrayed.
Finally, it is intended that both materials and dimensions may be varied according
to the user or device application requirements.

Method for separating particles of different types by difference in dielec-
trophoretic forces

It is assumed that the sample in the device chamber contains a mixture of particles
of at least two different types which are subject to negative dielectrophoresis and
positive dielectrophoresis respectively, at a given frequency. By energizing the elec-
trodes with periodic signals at that frequency, potential cages are established, into
which the particles of the first type are attracted and from which the particles of the
second type are repelled. Hence by moving the potential cages toward a separate
area of the device only the particle of the first type will be displaced. That area
may be, for example, a separate chamber in the device where particles of the first

type may be further collected, counted, mated with other particles etc.. It should be noted that in this case more than one particle per cage may be allowed.

Method for separating particles of different types by single-particle entrapment, type detection and motion

It is assumed that the sample in the device chamber contains a mixture of particles of at least two different types. It is further assumed that the size of the cages is such that only one particle may be trapped in each cage, and that each location on which the cages are established comprises a sensor able to detect the type of particle trapped in that cage, if any. This sensor may, for example, be of capacitive and/or optical type. After establishment of the dielectrophoretic potential cages, the particles in each cage are discriminated, and all cages trapping particles of one type are moved toward a separate area of the device so that only particles of that type will be present in that area. That area may be a separate chamber in the device where the particles may be further collected, counted, mated with each other or with other particles etc.. As used herein and in what follows, the term 'type' should be seen as referring to characteristics which may be discriminated by using sensors. In other terms, two particles made of the same matter, but of different size, may be regarded as belonging to different types if the sensor embedded in the device discriminates the two. Again, two particles made of different matter, but which cause the same output of the embedded sensor, may be regarded as belonging to the same type.

Method for separating particles of different types by single-particle entrapment, motion, type detection, and motion

This method is similar to the previous one, except for the fact that the locations on which the cages are first established need not comprise a sensor. Thus it is first necessary to displace particles -by moving cages -toward locations where a sensor is

able to detect their type, and then further displace the particles, according to their type, toward different areas of the device. These areas may be, for example, separate chambers in the device where the particles may be further collected, counted, mated with each other or with other particles, etc..

Method for counting particles of a type by single-type of particles entrapment and number detection

It is assumed that the sample in the device chamber contains a single type of particle, and that each location on which the cages are established comprises a sensor which is able to detect the number of particles trapped in that cage. This can be achieved if the output response of the sensor is proportional to the number of particles trapped in the cage associated. The total number of particles in the sample can be counted quite simply by summing the number of particles detected in each cage.

Method for counting particles of different types by single-particle entrapment and type detection

It is assumed that the sample in the device chamber contains one or more types of particle. It is further assumed that the size of the cages is such that only one particle may be trapped in each cage, and that each location on which the cages are established comprises a sensor able to detect the presence and type of the particle trapped in that cage, if any. Counting the number of particles of each type can thus be simply achieved by establishing potential cages, detecting the type of particle in each cage, if any, and separately summing the number of cages trapping particles of the same type.

Method for counting particles of different types by single-particle entrapment, motion and type detection

This method is similar to the previous one, except for the fact that the locations on which the cages are first established need not to comprise a sensor. Thus, it is first necessary to displace particles, by moving cages, toward locations where a sensor is able to detect their type. Then the type of any particle present in the cages at the sensing locations is detected. If other cages whose content has not yet been monitored are left over, the cage at the sensing location is displaced to allow cages whose content has not yet been detected to be displaced above the same sensing location. This last operation is repeated until the content of all e cages has been detected. Counting the number of particles of each type can therefore be achieved by separately summing the number of cages trapping particles of the same type.

CLAIMS:

1. Apparatus for the manipulation of one or more particles immersed in a fluid, comprising:

   – a first substrate;

   – a plurality of electrodes comprising a first electrode array realized on said first substrate and a second electrode array comprising at least one electrode, said second electrode array being faced toward and spaced apart from said first electrode array, said particles and said fluid being placed in a region between said first electrode array and said second electrode array;

   – means for applying a first electrical input to a first subset of said plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes

   said first electrical input and said at least one other electrical input establishing an electric-field having constant magnitude over at least one imaginary closed surface located entirely in said fluid.

2. Apparatus according to claim 1, wherein said second electrode array is realized on a second substrate.

3. Apparatus according to claim 1, wherein said first substrate includes sensing means for detecting the presence of one or more of said particles.

4. Apparatus according to claim 2, wherein said first substrate and/or second substrate includes sensing means for detecting the presence of one or more of said particles.

5. Apparatus according to claim 3 or 4, wherein said sensing means include electric-field measuring means for detecting variations in the electrical characteristics in at least a portion of said region between said first electrode array and said second electrode array.

6. Apparatus according to claim 5, wherein said electric-field measuring means include at least one electrode of said second electrode array and at least one electrode of said first electrode array, for detecting variations in the electric-field established between said at least one electrode of said second electrode array and said at least one electrode of said first electrode array.

7. Apparatus according to claim 5, wherein said electric-field measuring means include a first electrode of said first electrode array and at least one other electrode of said first electrode array, for detecting variations in the electric-field established between said first electrode and said at least one other electrode.

8. Apparatus according to claim 1, wherein said second electrode array is substantially transparent.

9. Apparatus according to claim 3 and 8, wherein said sensing means include optical-energy measuring means for detecting variations in the optical characteristics in at least a portion of said region between said first electrode array and said second electrode array.

10. Apparatus according to any one of the previous claims, further comprising means for changing said first electrical input and/or said at least one other electrical input for:

    − expanding or contracting, and/or

    − moving, and/or

  — establishing or deleting

said at least one imaginary closed surface

11. Apparatus according to any one of the previous claims, further comprising means for changing said first and/or said at least one other subset of said plurality of electrodes for:

  — expanding or contracting, and/or

  — moving, and/or

  — establishing or deleting

said at least one imaginary closed surface.

12. Apparatus according to any one of the previous claims, further comprising means to let said fluid flow into and/or out of said region between said first electrode array and said second electrode array.

13. Apparatus according to any one of the previous claims, further comprising a spacer interposed between said first substrate and said second electrode array, said spacer having at least one opening, said spacer forming at least one chamber between said first substrate and said second electrode array.

14. Apparatus according to any one of claims 1-12, further comprising a spacer integrated in said first substrate, said spacer having at least one opening, said spacer forming at least one chamber between said first substrate and said second electrode array.

15. Apparatus according to claim 2 or 4, further comprising a spacer integrated in said first substrate and/or said second substrate, said spacer having at least one opening, said spacer forming at least one chamber between said first substrate and said second electrode array.

16. Apparatus according to any one of the previous claims, wherein at least one electrode of said plurality of electrodes is connected to circuit means comprising:

— addressing input means;

— data input/output means;

— reference input means;

— at least one memory element;

whereby the electrical input applied to the electrode is derived from said reference input according to a value stored in said at least one memory element programmed by said addressing input means and said data input/output means.

17. Apparatus according to claim 16, wherein said circuit means further comprises sensing means.

18. Apparatus according to any one of the previous claims, wherein at least one of said electrodes of said first electrode array has rectangular shape.

19. Apparatus according to any one of the previous claims, wherein at least one of said electrodes of said first electrode array has hexagonal shape.

20. Apparatus according to any one of the previous claims, wherein said second electrode array consists of a single electrode.

21. Apparatus according to any one of the previous claims, wherein said first substrate is a monolithic semiconductor substrate.

22. Method for the manipulation of one or more particles immersed in a fluid, applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality

of electrodes, in order to establish an electric field having constant magnitude over at least one imaginary closed surface located entirely in said fluid, whereby said particles are either attracted toward or repelled from the region enclosed by said at least one imaginary closed surface depending on electrical properties of said particles and said fluid.

23. Method for the manipulation of one or more particles immersed in a fluid, comprising the steps of:

- applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes in order to establish an electric field having constant magnitude over at least one imaginary closed surface located entirely in said fluid, whereby said particles are attracted toward the region enclosed by said at least one imaginary closed surface ;

- displacing said at least one imaginary closed surface in order to attract said particles toward another region enclosed by said at least one imaginary closed surface .

24. The method of claim 23 wherein said displacing of said at least one imaginary closed surface is obtained by changing at least once said first subset of the plurality of electrodes and/or said at least one other subset of the plurality of electrodes.

25. The method of claim 23 wherein said displacing of said at least one imaginary closed surface is obtained by changing said first electrical input and/or said at least one other electrical input.

26. Method for the separation of different types of particles immersed in a fluid, comprising the steps of:

    — applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over at least one imaginary closed surface located entirely in said fluid, whereby the particles of at least one first type are attracted toward the region enclosed by said at least one imaginary closed surface and the particles of different types are repelled from said region;

    — displacing said at least one imaginary closed surface in order to move only said particles of at least one first type toward another region enclosed by said at least one imaginary closed surface.

27. Method for the separation of different types of particles immersed in a fluid, comprising the steps of:

    — applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over multiple imaginary closed surfaces located entirely in said fluid, whereby said different types of particles are attracted toward and trapped in the regions enclosed by said imaginary closed surfaces, each of said regions being able to trap only one particle;

    — sensing the type of each particle trapped in said regions;

    — displacing a first subset of said imaginary closed surfaces toward a first area, said first subset being composed of imaginary closed surfaces which trap particles of a first type, in order to move said particles of a first type toward said first area.

28. Method for the separation of different types of particles immersed in a fluid, comprising the steps of:

  - applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over multiple imaginary closed surfaces located entirely in said fluid, whereby said different types of particles are attracted toward the regions enclosed by said imaginary closed surfaces, each of said imaginary closed surfaces being able to trap only one particle;

  - sequentially displacing said imaginary closed surfaces toward at least one sensing location, in order to move trapped particles toward said at least one sensing location, and sensing the type of each particle in each of said at least one sensing location;

  - displacing toward a first area a first subset of said imaginary closed surfaces composed of those imaginary closed surfaces which trap particles of a first type, in order to move said particles of a first type toward said first area, and displacing toward a second area a second subset of said imaginary closed surfaces composed of imaginary closed surfaces which trap particles of different types, in order to move the particles of different types toward said second area.

29. Method for counting the number of particles of at least one type immersed in a fluid, comprising the steps of:

  - applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having

constant magnitude over at least one imaginary closed surface located entirely in said fluid, whereby only said particles of at least one type are attracted toward the regions enclosed by said at least one imaginary closed surface;

— sensing the number of particles in each of said regions.

30. Method for counting the number of particles of at least one type immersed in a fluid, comprising the steps of:

— applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over at least one imaginary closed surface located entirely in said fluid, whereby said particles are attracted toward the regions enclosed by said at least one imaginary closed surface, each of said regions being able to trap only one particle;

— sensing the presence and type of each particle in said regions;

— separately summing the number of particles of the same type.

31. Method for counting the number of particles of at least one type immersed in a fluid, comprising the steps of:

— applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over multiple imaginary closed surfaces located entirely in said fluid, whereby said particles are attracted toward the regions enclosed by said imaginary closed surfaces, each of said regions being able to trap only one particle;

– sequentially displacing said imaginary closed surfaces toward at least one sensing location, in order to move the trapped particles toward said at least one sensing location, sensing the presence and type of each particle in each of said at least one sensing location;

– separately summing the number of particles of the same type.

32. The method of any one of claims 26, 27, 28, 31 wherein said displacing of the imaginary closed surfaces is obtained by changing said first subset of the plurality of electrodes and/or said at least one other subset of the plurality of electrodes.

33. The method of any one of claims 27, 28, 29, 30, 31, wherein the detection is obtained by measuring variations in the electrical and/or optical characteristics in at least a portion of said fluid.
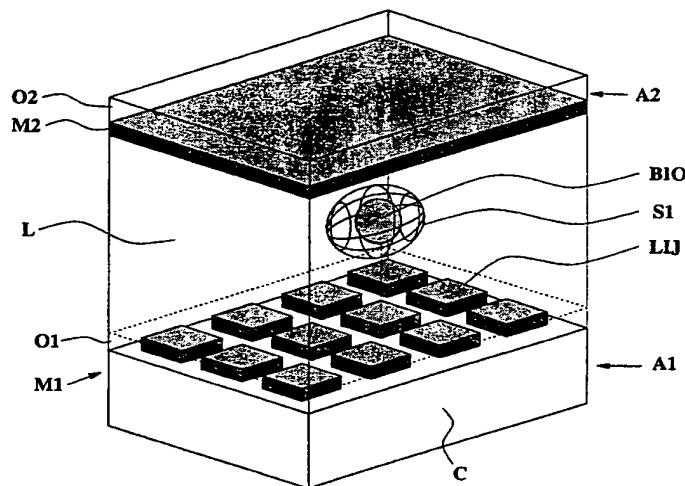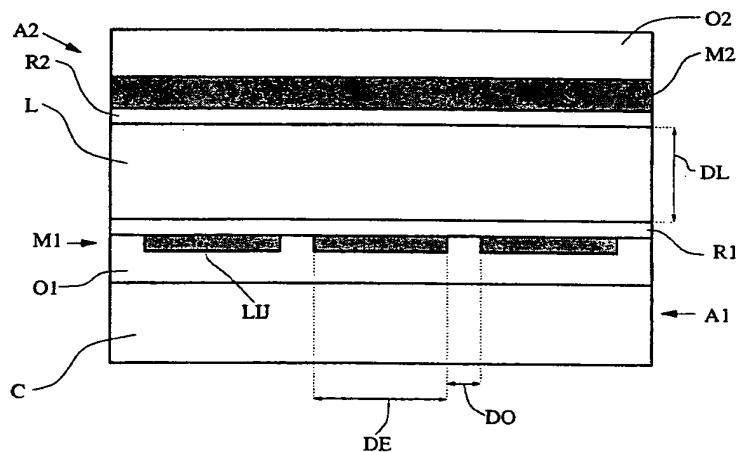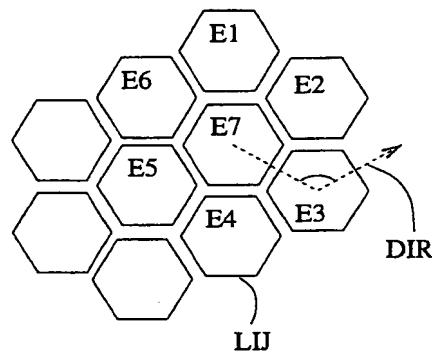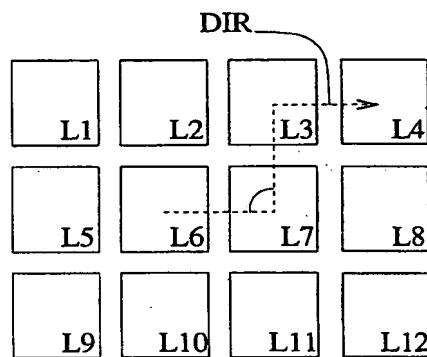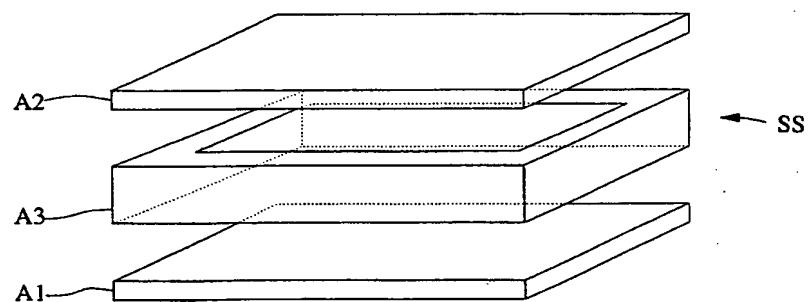
Figure 1:

Figure 2:

Figure 3:

Figure 4:

Figure 5:
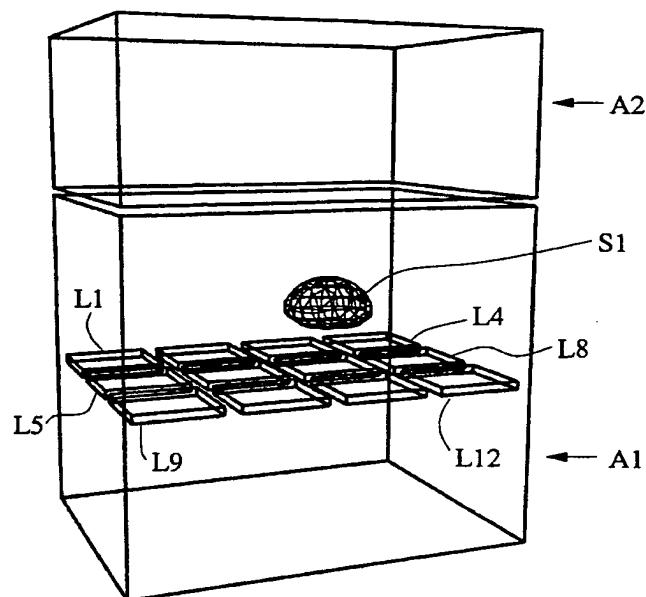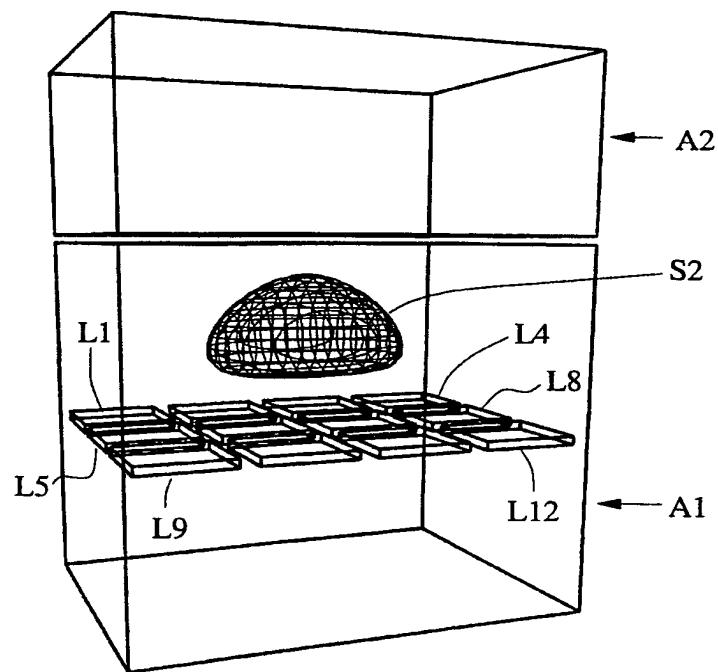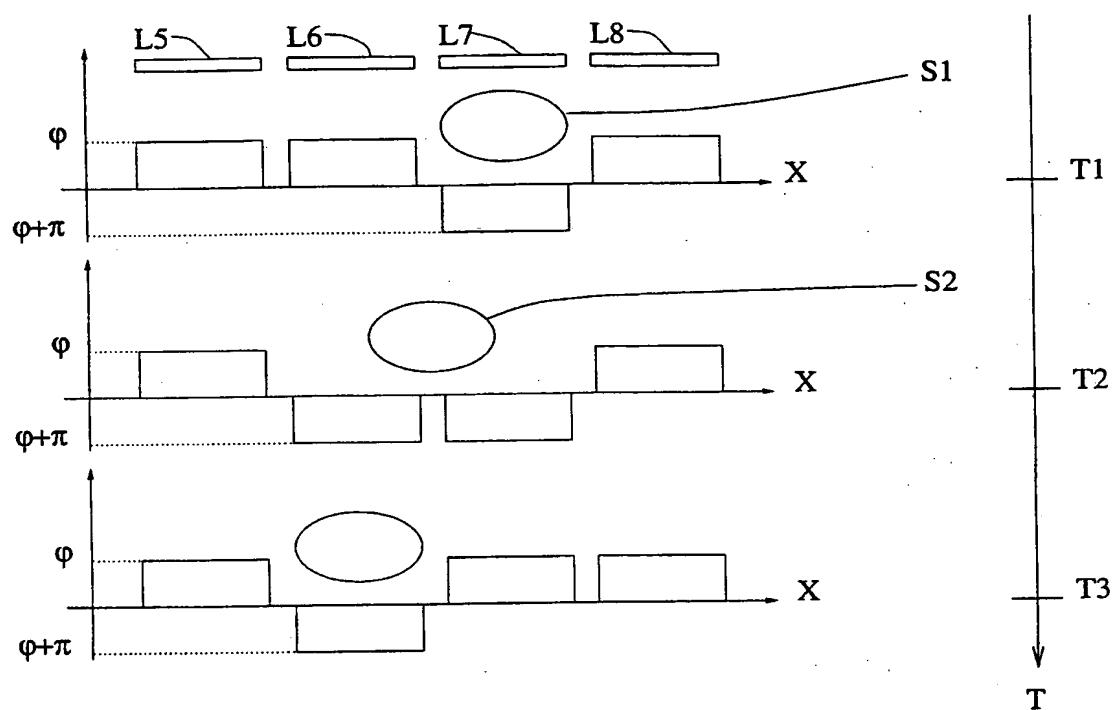
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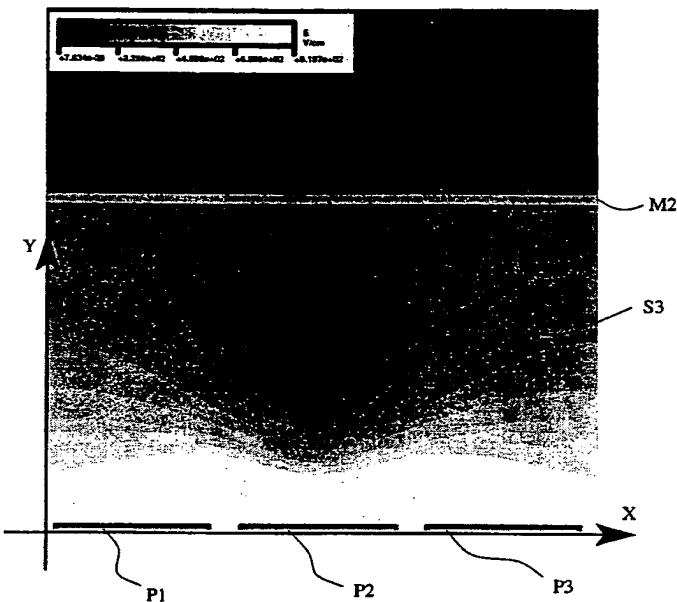
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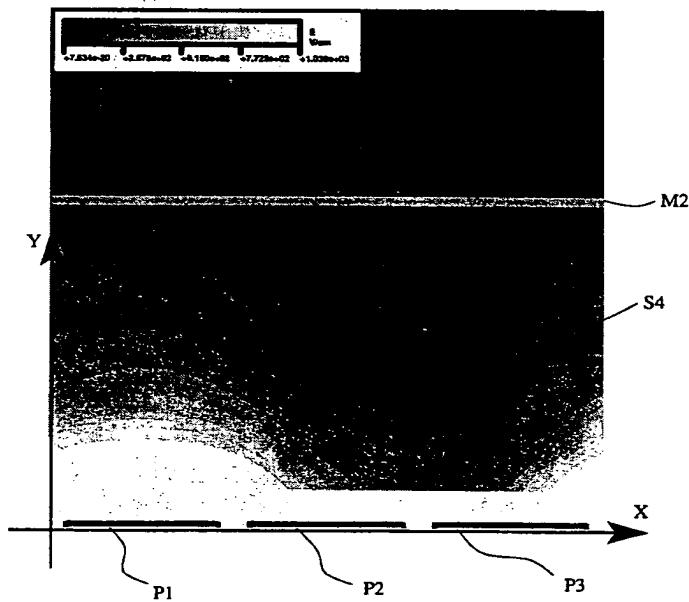
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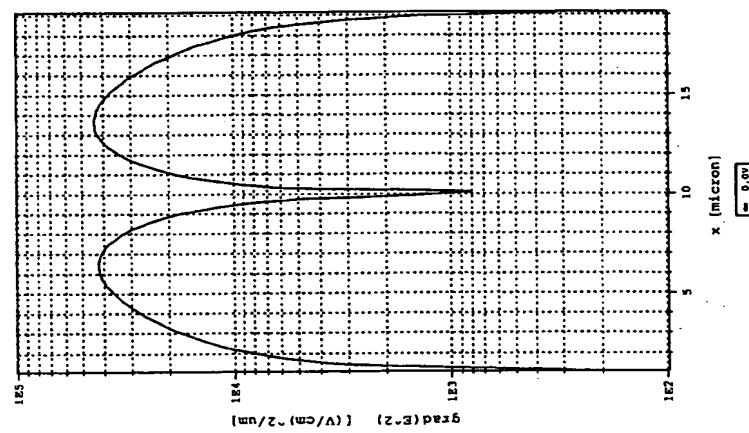
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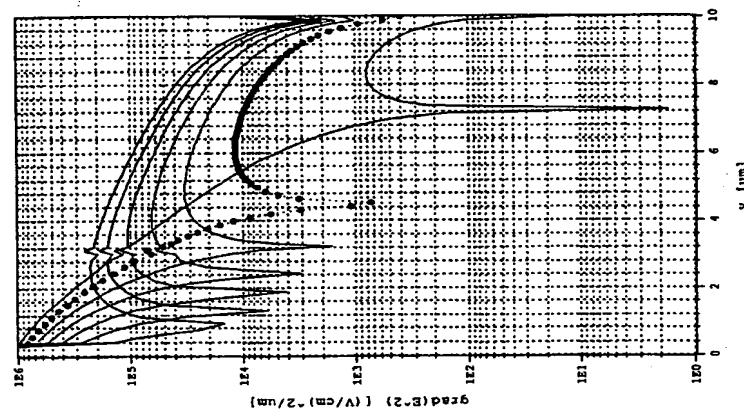
Figure 10:

Figure 11:

Figure 12:
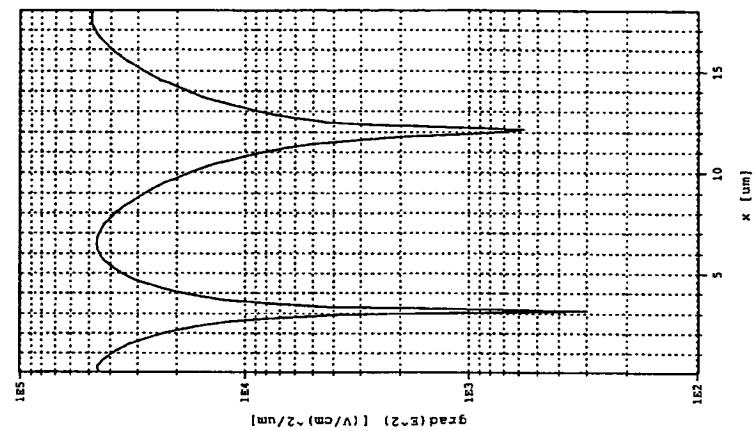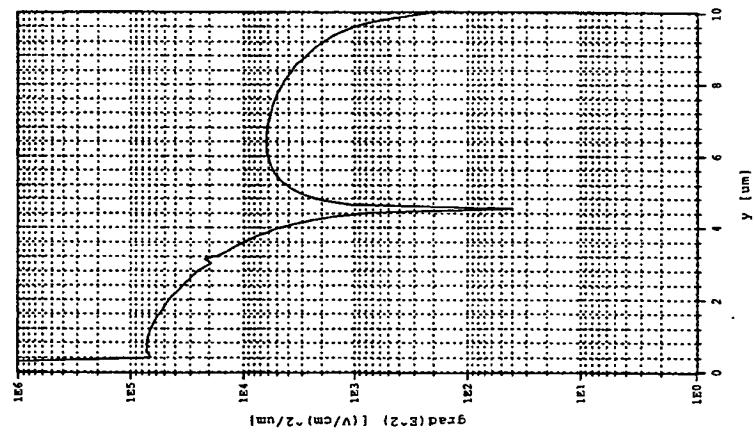
Figure 13:

Figure 14:
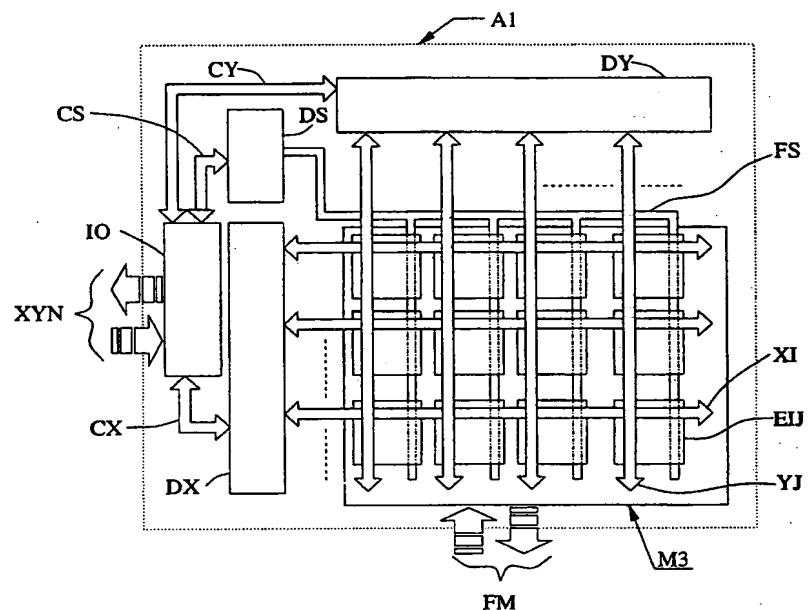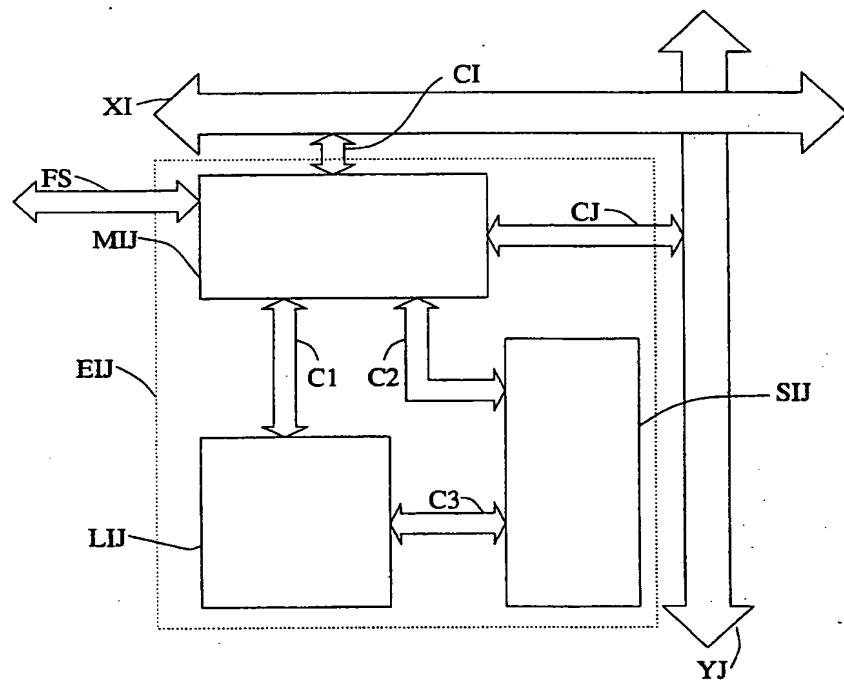
Figure 15:

Figure 16:
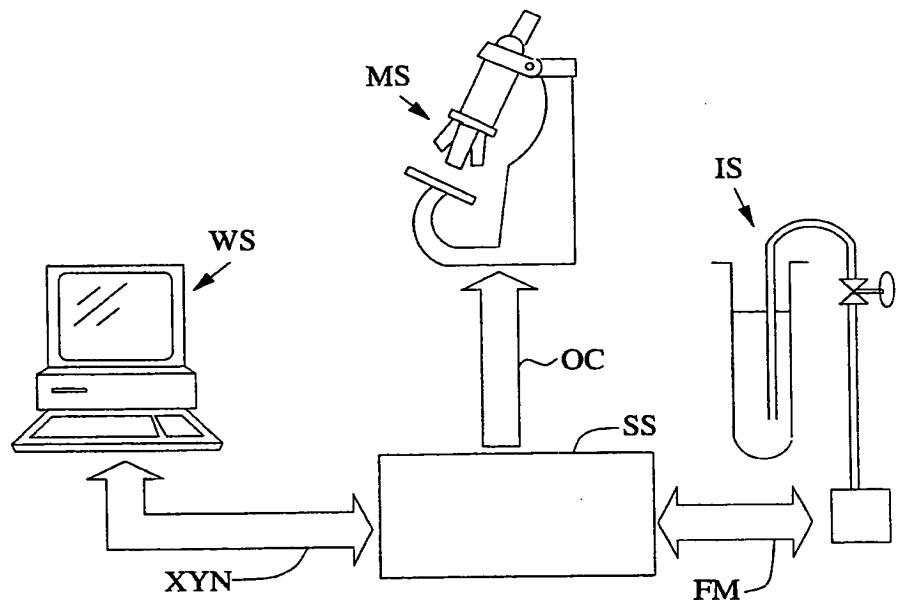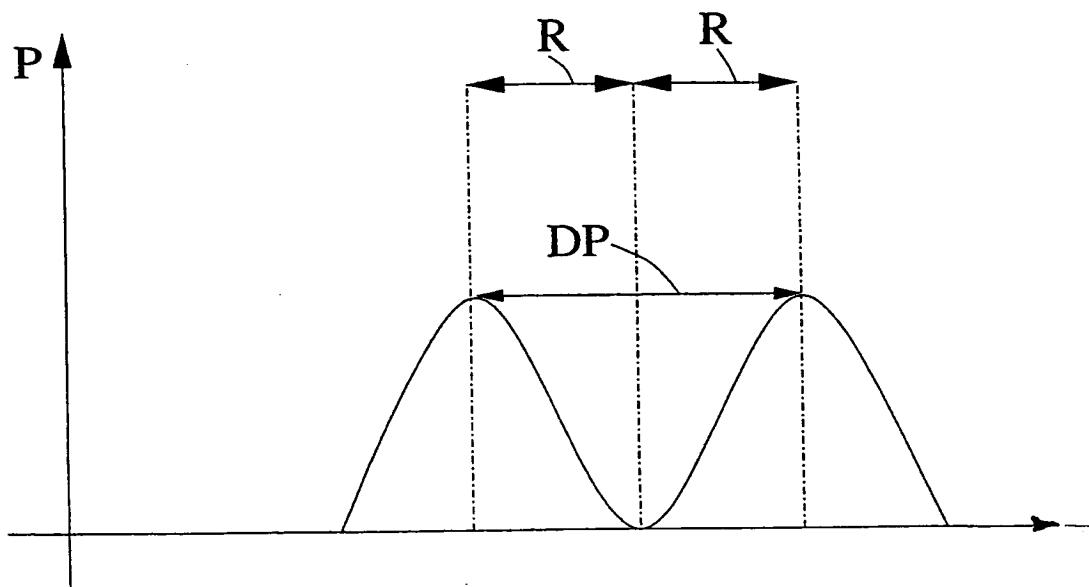
Figure 17:

Figure 18:
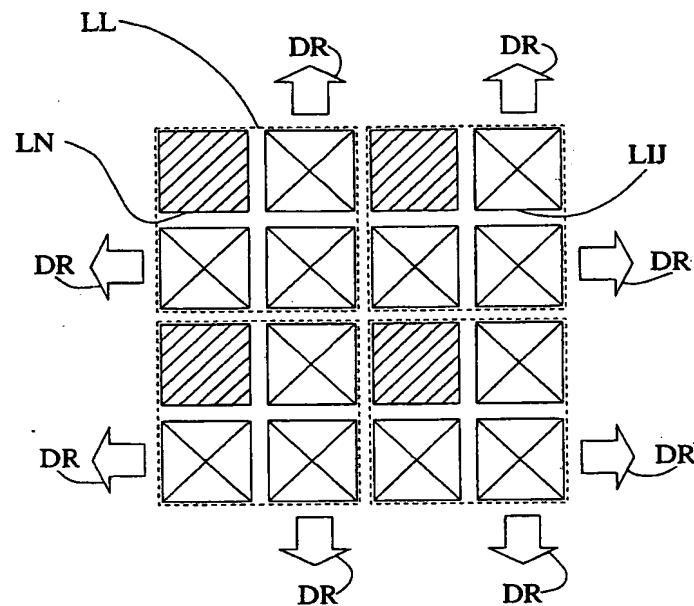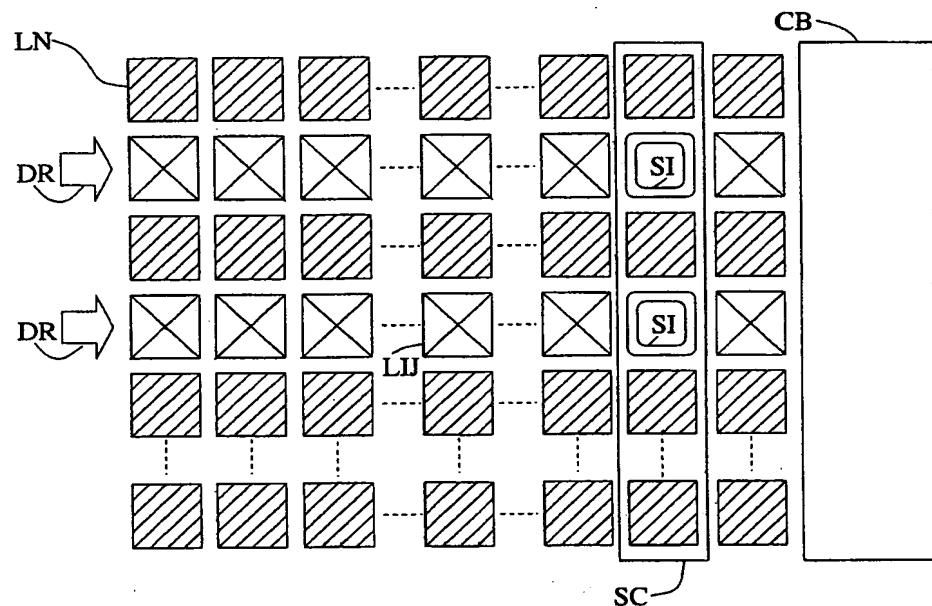
Figure 19:

Figure 20:
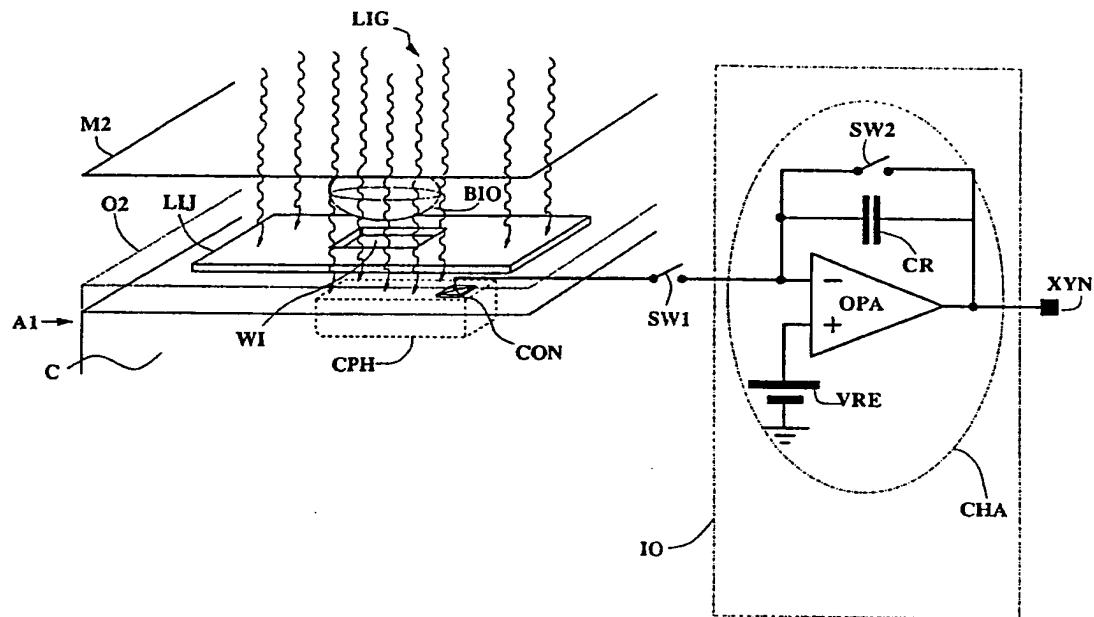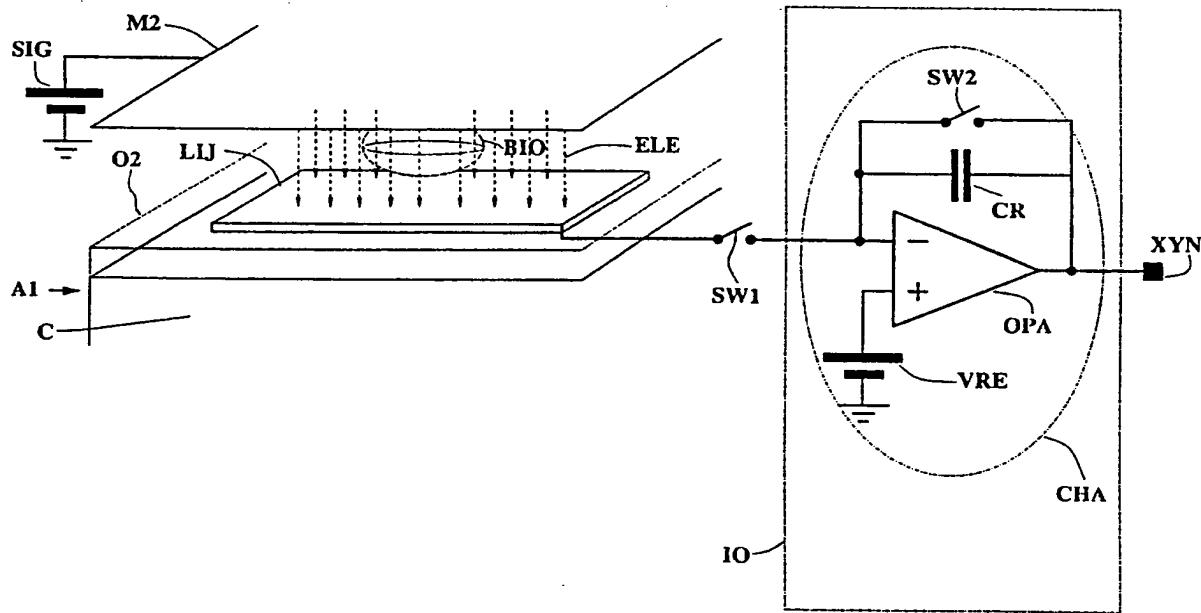
Figure 21:

Figure 22:
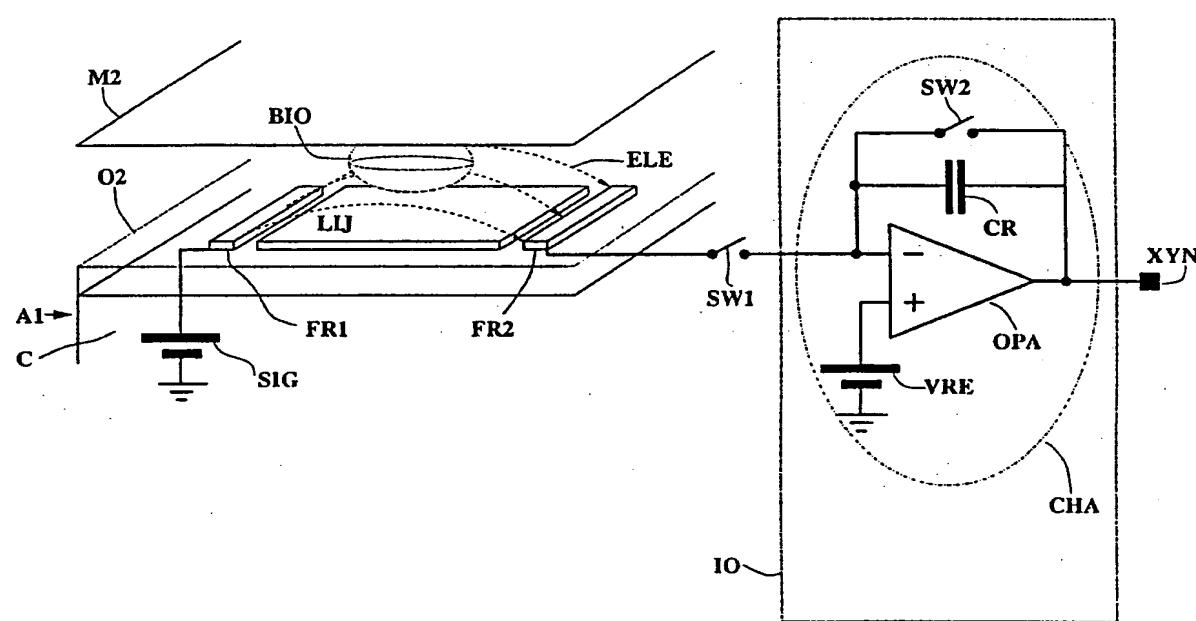
Figure 23:

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   B03C5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   B03C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FUHR G ET AL: "POSITIONING AND MANIPULATION OF CELLS AND MICROPARTICLES USING MINIATURIZED ELECTRIC FIELD TRAPS AND TRAVELLING WAVES" SENSORS AND MATERIALS,JP,SCIENTIFIC PUBLISHING DIVISION OF MYU, TOKYO, vol. 7, no. 2, 1995, pages 131-146, XP000617993 ISSN: 0914-4935 page 131 -page 134, paragraph 1; figure 4 -/--	1,2,10, 12-15, 21-23,25

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 August 2000	23/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Decanniere, L

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INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SCHNELLE T ET AL: "THREE-DIMENSIONAL ELECTRIC FIELD TRAPS FOR MANIPULATION OF CELLS - CALCULATION AND EXPERIMENTAL VERIFICATION" BBA - GENERAL SUBJECTS,NL,ELSEVIER SCIENCE PUBLISHERS, vol. 1157, no. 2, 1993, pages 127-140, XP000614981 ISSN: 0304-4165 cited in the application the whole document	1,2,8, 12,13, 16,18, 22,26
A	WO 99 17883 A (CALIFORNIA INST OF TECHN) 15 April 1999 (1999-04-15) claims 1,3-5,9,33,34	1,2,10, 22,23,25
A	FIEDLER S ET AL: "ELECTROCASTING - FORMATION AND STRUCTURING OF SUSPENDED MICROBODIESUSING A.C. GENERATED FIELD CAGES" MICROSYSTEM TECHNOLOGIES,DE,BERLIN, 1 December 1995 (1995-12-01), pages 1-7, XP000199394 ISSN: 0946-7076 figure 2	1,8,9
A	WO 91 08284 A (NAT RES DEV) 13 June 1991 (1991-06-13) claims 1,3,5	1,8,9

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